3247-Pos Board B352
Novel Mechanisms of Cell Uptake in Lipid-Mediated Gene Delivery
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The mechanism of cell uptake in lipid mediated gene delivery was investigated in NIH3T3 and CHO cell lines. We show that different endocytic pathways are activated by shape coupling between lipoplex and membrane lipids. Our results suggest that tailoring the lipoplex lipid composition to the patchwork-like plasma membrane profile could be a successful machinery of coordinating the endocytic pathway activities and the subsequent intracellular processing. Transfection experiments performed at 4°C, when endocytosis does not take place, show that a novel class of highly efficient multicomponent lipoplexes enter cells by a temperature-independent fusion-like mechanism. In vivo, plasma proteins bind to lipoplex surface and create a rich 'protein corona' that is recognized by cells and other biological structures. The 'protein corona' associated to lipoplexes after interaction with human plasma was found to be much richer in basic immunoglobulins gamma proteins (Ig-Gs) than that of pure lipid vesicles in the absence of DNA. Because surface properties of lipoplexes may determine their interaction with cells and tissues, an accurate knowledge of lipoplex surface properties may be important for predicting biological responses. These findings also suggest the existence of hybrid structures made of multilamellar complexes either stuck together by DNA or coexisting with DNA-loaded intact vesicles.

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Lipid Trafficking in Neurons and Schwann Cells
James Matthew Love, Gunja Dave, Joshua Chetta, Sameer Shah.
Axons in the peripheral nervous system are capable of extending thousands of times the length of their cell bodies. These projections possess high surface area to volume ratios and thus require large quantities of lipids to maintain their membrane structure. During initial development this demand for lipids can be quite high as axons are capable of extending at rates of 1 mm/day. Studies suggest that long distance transport of lipids occurs in membranous vesicles with insertion occurring only at the growth cone. Other sources of membrane lipids have been demonstrated including locally synthesized lipids in axons, transfer of lipids from myelin, and addition of lipids internalized along the axon. Robust analysis and quantification of lipid trafficking has yet to be well characterized.
To quantify these phenomena, a model of developmental sensory axons utilizing dorsal root ganglia (DRG) of day 1 to 5 rats were used. Cells were harvested and plated overnight. Cultures were then treated with cytosine arabinoside to remove fibroblasts. Two days post-harvest, cultures were treated with a fluorescent ceramide analogue labeled with boron dipyrromethene difluoride (BODIPY). One hour post-treatment the cultures were observed for twenty minute time intervals using fluorescent microscopy. Preliminary results indicate multiple transport processes may play a role in the trafficking of lipids in the axons of neurons. At least two distinct populations seem to exist; one composed of bright, punctuate fluorescence that may be attributable to vesicles and a second diffuse fluorescence that may be attributable to labeled plasma membrane lipids. Quantification of the traffic patterns seem to initially suggest the bright, punctuate population are more motile than the diffusely fluorescent populations. Future experiments will compare patterns observed in neurons with those in Schwann cell projections, which may be a source of lipids transferred to axons.

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mRNA Transport in the Projections of Maturing Hippocampal Neurons
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Translation of mRNA in axons and dendrites enables a rapid supply of proteins to specific sites of localization within the neuron. Distinct populations of mRNA-containing cargoes, including granules and mitochondrial mRNA, are transported with neuronal projections. The distributions of these cargoes appear to change during neuronal development, but details on the dynamics of mRNA transport during these transitions remain to be elucidated. The goal of this project is to characterize transport of mitochondrial and non-mitochondrial mRNA in neuronal projections during the development of hippocampal neurons. One day old rat hippocampal neurons were cultured for 1, 5, and 7 days, and mRNA transport was examined at each time point. The transport was observed via real-time imaging of SYTO14, a fluorescently labeled marker for mRNA. To differentiate between mitochondrial and granular mRNA, cells were also labeled with MitoTracker. Quantitative analysis was performed by kymograph which gives a graphical representation of spatial position over time. The results suggest differences in the transport pattern of mitochondrial and non-mitochondrial mRNA, and also indicate significant differences in transport parameters at different time points. Unexpectedly, increased bidirectional velocity of mRNA transport was observed from day 1 to day 7, which suggests altered demand for locally synthesized proteins even after maturity. To better understand the logic underlying altered mRNA transport, we are currently investigating whether changes in transport correlate with specific stages of neurite differentiation into axons or dendrites or synaptic contact with other neurons. This work has important implications for the regulation of neuronal plasticity during neuronal growth, maturity, and neurodegeneration.

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Microtubule Motors cannot Coordinate Bidirectional Transport of Lipid Droplets in the Absence of Cytosolic Components
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Long-range intracellular cargo transport is achieved by kinesins and cytoplasmic dyneins each of which moves mostly unidirectionally along microtubules. Similar to many other cargos, lipid droplets in *Drosophila* embryos exhibit global directionality with local bidirectional motion resulting from a biased engagement of the opposite polarity motors. Using Differential Interference Contrast microscopy, we examined purified lipid droplets from *Drosophila* embryos *in vitro*. Unlike lipid droplets *in vivo*, a large fraction of the purified droplets exhibit short distance transport. This suggests that, in the absence of cytosolic non-motor proteins, transport is impaired leading to opposite polarity motors engaging in a tug-of-war. This stalemate is consistent with previous *in vivo* force measurements that demonstrated equal force generation in both directions. Force measurements using an optical trap show that the motors on the purified lipid droplets are functional. We specifically alter this activity for the opposite polarity motors to resolve the stalemate.

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Dynamic Behavior of Intracellular Vesicles Probed with Two-Color Single Particle Tracking
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Understanding the dynamics of intracellular vesicles is essential for studies of cellular internalization, intracellular transport, and the movement of proteins between organelles. Of particular interest are the dynamics that result in the fusion of vesicles as extracellular cargo is transported through the endocytic pathway. We use two-color single particle tracking fluorescence microscopy to study populations of vesicles that exist as both isolated vesicles and fused hybrid vesicles. Specifically, we are interested in the relationship between Rab7-vesicles, LAMP1-vesicles, and Rab7-LAMP1-hybrid vesicles. The majority of these vesicles exist as hybrid vesicles, which limits the ability of static measurements and standard biochemical approaches to resolve differences between these three populations. We propose that while these vesicle populations are highly colocalized, they can be distinguished by their dynamics during the short periods when they are not colocalized. To this end, individual vesicles, labeled with distinct variants of GFP, were tracked and analyzed. An algorithm was developed to obtain the total and linear distance traveled by the vesicle, travel direction relative to its eventual fusion site, and efficiency in reaching its destination. Significant differences in these measurements suggest LAMP1-vesicles fuse more efficiently than Rab7-vesicles. This approach will prove useful in future studies for resolving the differences in highly colocalized populations of vesicles.

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Superresolution Studies to Reveal the Interactions between Motor Proteins and Individual Cargos in Chlamydomonas Flagella
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Our research aims to understand the molecular mechanism of cytoplasmic dynein, which is involved in the transport of cargo towards the microtubule minus end of eukaryotic cells. Specifically, we use intraflagellar transport (IFT) in Chlamydomonas cells as a model system to study interaction of IFT dynein with kinesin II, opposite polarity motor that moves cargos toward the plus end. To detect the distribution and cargo interaction of single motor proteins along the flagellum, we employed Stochastic Optical Reconstruction Microscopy (STROM) microscopy. This technique has proven to construct superresolution images of precisely positioned fluorophores from single-molecule images. In order to facilitate STORM imaging, photoswitchable cyanine reporter and an activator molecule were coupled to antibodies against kinesin II and IFT dynein. The quantitative analysis of the STORM images will allow us to determine how many kinesin and dynein motors actively work on a cellular cargo, and how the cargo direction is determined by kinesin-dynein interactions. IFT is a universal process in all eukaryotic cilia and flagella. Defects in this process are the primary causes of polycystic kidney disease and retinal degeneration.